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Antibacterial Activity OF Methanolic Extract of *Syzygium alternifolium* Leaves

M. Sangeeth Kumar*, Nusrath Yasmeen

Department of Pharmacology, University College of Pharmaceutical Sciences, Kakatiya University, Warangal, India.

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Address for Correspondence
Department of Pharmacology, University College of Pharmaceutical Sciences, Kakatiya University, Warangal, India..
Tel. +91-9885606110.
E-mail: octobertwentyseven@rediffmail.com

ABSTRACT

syzygium alternifolium is a member of Myrtacea family, is used for curing various ailments according to relevant traditional approaches on the plant drugs therapy. The present study was carried out to evaluate the antimicrobial properties of the methanolic, petroleum ether, ethylacetate, chloroform, benzene extracts of *syzygium alternifolium* leaves on certain pathogens. The methanolic, benzene, and petroleum ether showed significant results whereas the chloroform extract did not show any antimicrobial activity on any pathogen, also all these extracts were in significant on aspergillus niger. It was observed that as the disc dosage level increases the inhibitory effect also increased. These findings justify the use of the plants for the study of antibacterial activity. Their toxicity level and antimicrobial activity with different extraction solvents should further be studied to use them as potential sources and templates for the synthesis of drugs to control infectious diseases.

Keywords: *Syzygium alternifolium*, Disc Diffusion method, anti microbial activity, zone of inhibition.

INTRODUCTION

There are many traditional systems of medicine in the world, each with different associated philosophies and cultural origins. Some of these such as Tibetan traditional medicine remain relatively localized in their country of origin, while others such as Ayurvedic and Chinese traditional medicines are increasingly used in many different areas of the world. Herbal care or traditional system of medicine are used throughout the world and from century's herbs have been the original source for most of the drugs. The anti bacterial activity has been screened in many plants because of its great medicinal relevance with the recent years, infections have increased to a great extent and resistance against antibiotics became ever increasing therapeutic problem. Due to indiscriminate application of antibiotic drugs most of the microbial organisms' high resistance to a good number of commercial antibiotics. This coupled with other problems like the dangerous side effects of some commercial antibiotics have

led the scientists to think of other alternatives like new antimicrobial substitutions from other sources especially in plants .the presence of antimicrobial substances in the higher plants is well established fact and they provide a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health. The antimicrobial compounds from plants may inhibit microbial growth by different mechanism than those presently used antimicrobials and may have a significant clinical value in treatment of resistant microbial strains[1-5].

syzygium alternifolium(Wt.)Walp is a member of Myrtaceae family, is an endemic aromatic tree, distributed in Assam and Andhrapradesh of India. Locally it is known as mogi/movi. The plant parts were used in traditional medicine to cure various diseases viz.,tender shoots and fruits for dysentery, seeds for diabetes and stem bark was used to treat gastric ulcers[6.] *Syzygium alternifolium*, was reported to possess hypoglycemic and antihyperglycemic activity and contain flavonoid constituents. Based on review of literature, no reports are available regarding antimicrobial property *of syzygium alternifolium* leaves. Hence the present study gains importance to screen the antimicrobial properties of *syzygium alternifolium*[7,8].

Antibiotics provide the main basis for the therapy of microbial infections. Since the discovery of these antibiotics and their uses as chemotherapeutic agents there was a belief in the medicinal fraternity that this would have the eventual eradication of infectious diseases. The di–C-methylflavone was isolated from leaves of *Syzygium alternifolium* which has been identified as 4, 5–dihydroxy-7-methoxy-6,8-dimethyl-flavones (sideroxylin).

Only four C-methyl flavones, strobochrysin, 5-hydroxy-7, 4'dimethoxy-6 methoxy-flavone, eucalyptin and sideroxylin were identified.

MATERIALS AND METHODS

Plant Material

The leaves of *syzygium alternifolium* were collected during winter season from Tirumala hills, Tirupathi, AP, and India. It was identified and authenticated by Prof, Madhava chetty, K., Taxonomist, S.V. University, Tirupathi, A.P, India. A voucher specimen has been kept in our lab for future references.

Preparation of extract and paper discs

The collected leaves were shade dried, powdered and extracted with various solvents by the method of maceration for 10 days. The practical yield was found to be 18.7%.The extract was then filtered and the filtrate was concentrated under reduced pressure at 40^{o}C using a rotoflash evaporator. The solvents used were methanol, benzene, ethylacetate, petroleum ether, chloroform, the respective extracts were then subjected to antibacterial screening against the pathogens. Sterilized Whatman No.1 filter paper discs of 6mm diameter were saturated with 20µl of the extract and allowed to dry at room temperature in laminar air flow bench.

Qualitative anti bacterial studies[11-13]

Method followed

Disc diffusion method[9]

Working procedure

Preparation of test and standard solution: the test solution of various extracts of *S.alternifolium* was prepared in distilled water at a concentration of 10mg/ml. Streptomycin was used as standard which was also dissolved in distilled water to get a final concentration of 30µg/ml .

Microorganism used

The microbial strains viz., Enterococcus, S.aureus MTCC 737, E.coli MTCC 1687, P.aeruginosa MTCC1688, Proteus vulgaris, Penicillium notatum, were used to test the extracts .These organisms were obtained from the microbial type culture collections centre Dept., of Microbiology., Deccan college of medical sciences., A.P. The organisms were stored on agar slant in Mccartney bottles and kept in the refrigerator, prior to subculture.

Culture medium

The following media were used for the antimicrobial studies;

Nutrient Broth

37gm of readymade powder was dissolved in 1ltr of distilled water.

P^H adjusted to 7.8 and stabilized by autoclaving at 15 lbs for 15min.

Nutrient Agar

The sterilized medium was cooled to 40^{o}C and poured into petri dishes to obtain 4-6mm thickness the media was allowed to solidify at R.T.

Antibacterial assay procedure

A sterile borer was used to prepare cups of 10mm diameter in the agar media spread with the microorganism. 0.1 ml of inoculums (of 10^4 to 10^6 CFU/ml population prepared from standardized culture, adjusted with peptone water fixed by optical density (OD= 0.008&0.1) (10) was spread on agar plate by spread plate technique. Accurately measured (0.1ml) solution of each sample and standard samples were added to the cups with a micropipette .All plates were kept in refrigerated at 2-8^{o}C for a period of two hours for effective diffusion of test compounds and standard. Later the Petri plates were incubated at 37ºC for 24 hrs in BOD incubator and the diameter of the zone of inhibition were measured in millimeter. The efficacy of extracts against clinically important bacteria was compared with standard antibiotic streptomycin (30ug/ml) which showed a good zone of inhibition against the tested organism. Strategies of comparison are given in the Table 1.the results are tabulated in Table 2 and Fig 1.

Qualitative anti fungal studies[14-20]

The *in-vitro* antifungal activity by Disc diffusion method was standardized using Fluconazole. This method is based on diffusion of antifungal component from reservoirs hole to the surrounding sabourads dextrose agar medium, so that the growth of fungus is inhibited as Zone around the hole. Two fungi were selected Viz., Aspergillus niger and Candida albicans MTCC 183.

Fungi used

Standard cultures of Candida albicans and As pergillus niger were employed for the present study. The fungi were maintained by subculturing and used at regular intervals.

Sample Preparation

Samples were dissolved in distilled water to get concentration of 30µg/ml.

Culture medium

Sabourad dextrose agar medium (Himedia) was used for preliminary antifungal activity .The medium was prepared by dissolving in water and autoclaving at 121^{o}C for 15 min.

Standard preparation

Fluconazole standard was purified at fixed concentration of 10µg/ml in sterile distilled water.

Working procedure

An inoculum was suspending a single isolated colony in about 5ml of 0.95 w/v of normal saline. This was mixed slowly to

achieve a smooth suspension later one drop of Tween -20 was added for filamentous fungi and the mould was broken by shaking .A sterile cotton swab was moistened in the inoculums suspension and excess was removed by rolling the cotton swab on the inside of the tube, above fluid level 30 ml of sterile hot Sabourad agar medium was poured in each plate and allowed to harden on a level surface. A glass spreader was moistened in the adjusted inoculums suspension and surfaces of sabourand dextrose agar plates were streaked in four different directions (at 90^{o} angles). So as to cover the entire surfaces using sterile borer the medium was bored and the prepared test samples of three concentrations were taken and 0.1ml each test sample was added in each bore. This powder was carried out for the both fungi viz., C.albicans and A.niger. The surface of sabourad agar plate was dried out at 35^{o}C. Bore were made using sterile cork borer. The above operation was carried out in aseptic conditions and 0.1ml test solution was added to the respective bore and 0.1ml fluconazole was taken as standard reference. The plates were incubated at 35^{o}C for 48hrs. Later the values of zones of inhibition were recorded.

RESULT AND DISCUSSION

The weight of the dry residue obtained was 37gms and % yield calculated to be 18.5%.Data on invitro antimicrobial properties of *S. alternifolium* were depicted in Table2. present observations revealed that the test extracts of *S.alternifolium* possessed good antimicrobial activity against both bacteria and fungi. Among the test extracts of S. alternifolium, petroleum ether extract exhibited minimum inhibition zones(9-13mm) against all the pathogens ,except S.aureus and C.albicans while ethyl acetate and methanolic extracts showed moderate inhibition zones (6-10mm) In present investigation , gram negative bacteria ,E.coli, K.pneumonia were more susceptible than Gram positive bacteria , Bacillus cereus,and S.aureus . Two flavonoids were isolated viz., eucalyptin and tephrowatsin and a terpenoid, friedlin from *S.alternifolium* . Eucalyptin was reported to possess antimicrobial activity [21] while friedelin reported to possess antileishmanial activity[22]. So the broad spectrum of antimicrobial activity observed in present study appeared to be due to the individual or combined effect of the above mentioned chemical constituents.

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CONCLUSION

The results obtained in this study showed that the folkloric use of this plant in some of the above mentioned conditions is justified .the findings in this research reveal that S.alternifolium is another promising antimicrobial plant of myrtacee family which needs further studies to reveal the pharmacological potentials of the secondary metabolites present in the plant.

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Table 1

Zone size	Interpretation
Equal to wider than or not more than 3 mm smaller than the control(Amoxicillin)	Susceptible
Zone size greater than 3 mm, but smaller than the control by more than 3 mm	Intermediate
Zone sizes 3 mm or less	Resistant

Table 2. Antimicrobial screening of *Syzygium alternifolium*-leaves Zone of inhibition (in mm)

	M*(mg/ml)	C*(mg/ml)	B*(mg/ml)	E*(mg/ml)	P*(mg/ml)	S
Organisms						30
Staphylococcus aureus	8	0	4	0	5	23A
Escherichia coli	0	0	4	0	7	22A
Pseudomonas aeruginosa	6	0	0	15	3	28A
Aspergillus niger	0	0	0	0	0	23F
Candida albicans	4	0	3	3	9	25F
Pencillium notatum	0	0	8	5	10	22A
Enterococcus	2	0	6	6	6	23A

*Extracts: P: petroleum ether; E: ethyl acetate; M: methanol; C-chloroform; B-benzene
S: standard antibiotics; A: streptomycin; F: Fluconazole; standards concentration 30µg/disc

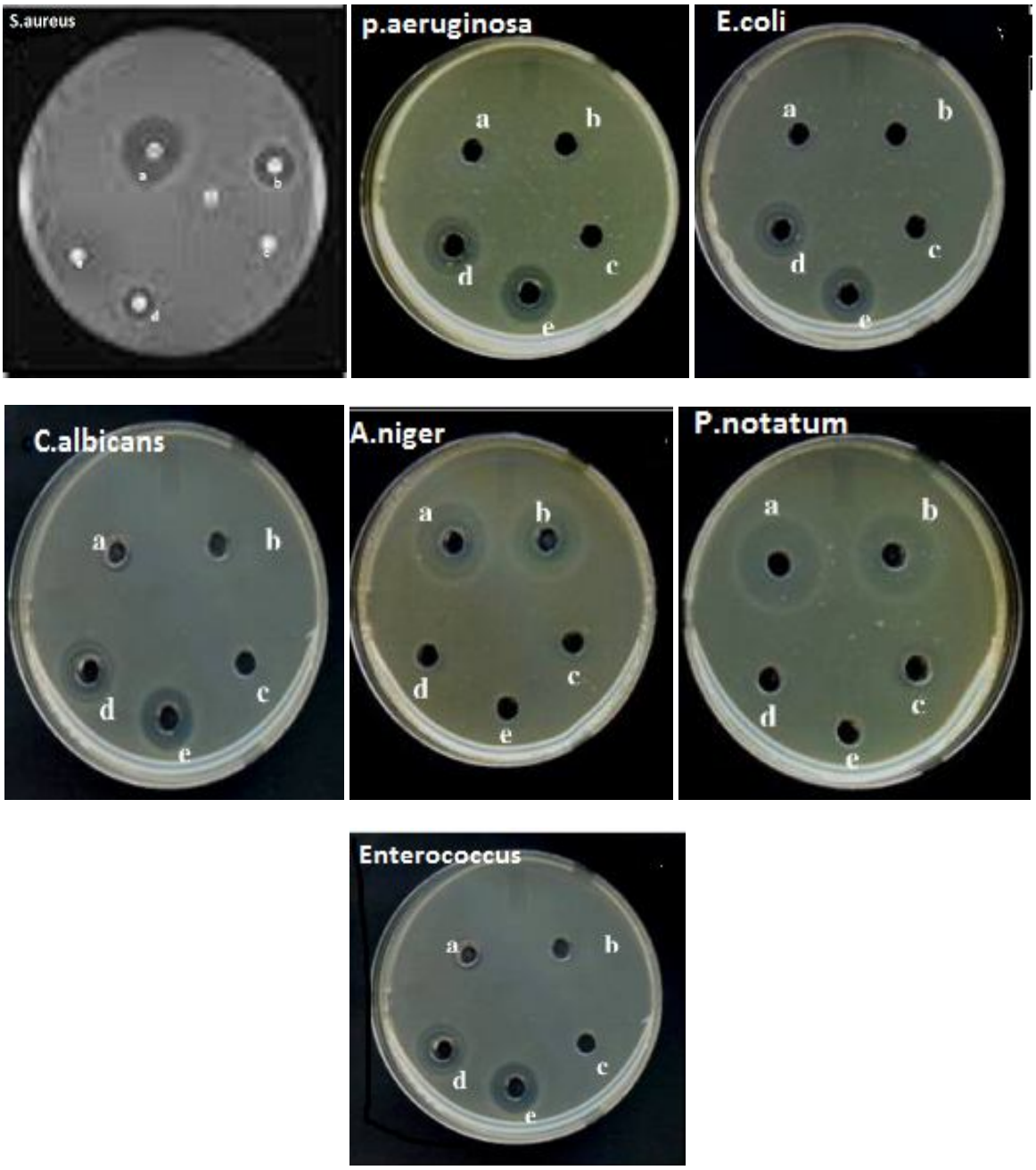

Figure 1. Antimicrobial screening of Syzygium alternifolium-leaves Zone of inhibition (in mm)
*Extracts: **P**: petroleum ether**; E**: ethyl acetate**; M**: methanol; **C**-chloroform; **B**-benzene
S: standard antibiotics; **A**: streptomycin; **F:** Fluconazole; standards concentration 30µg/disc